Disease Activity, Oxidized-LDL Fraction and Anti-Oxidized LDL Antibodies Influence Cardiovascular Risk in Rheumatoid Arthritis*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Patients with rheumatoid arthritis (RA) have a shortened lifespan compared to the general population. The high rate of premature mortality in the RA population can be attributed to cardiovascular disease (CVD).

Objectives. The aim of the study was to look for non-classic risk factors that can at least partially explain the enhanced cardiovascular (CV) risk in patients with RA.

Material and Methods. This was an observational study with 37 RA patients and 24 healthy volunteers as controls. The participants’ medical history was taken, and systematic coronary risk evaluation (SCORE) and carotid ultrasonography examinations were performed on all the participants. Laboratory tests included antibodies anti-cyclic citrullinated peptide (anti-CCP), inflammatory markers, lipid level, oxidized low-density lipoprotein (oxLDL) level and the level of anti-oxLDL antibodies.

Results. Both SCORE and oxLDL fraction were elevated in RA patients as compared to the healthy controls (3.1 ± 3.7 vs. 0.8 ± 1.2, p = 0.005; and 0.029 ± 0.033% vs. 0.014 ± 0.006%, p = 0.04, respectively). In the RA group, the presence of anti-CCP was associated with thickening of the carotid intima-media complex and SCORE elevation. In the RA group, significant correlations were found between SCORE and mean carotid intima-media thickness (IMT; RP = 0.34, p = 0.040), disease activity score (RP = 0.42, p = 0.011), erythrocyte sedimentation rate (ESR; RP = 0.35, p = 0.036), and disease duration (RP = 0.52, p = 0.002). In RA patients with carotid plaques, the oxLDL fraction was significantly elevated in comparison to those without plaques (0.055 ± 0.070% vs. 0.022 ± 0.018%, p = 0.033). In the RA group, there was a significant negative correlation between mean carotid IMT and the serum concentration of anti-oxLDL antibodies (RP = -0.38, p = 0.02). No association was noted between the presence of rheumatoid nodules and SCORE or carotid IMT.

Conclusions. Among RA patients, disease activity, ESR, disease duration, the presence of anti-CCP antibodies, the oxLDL fraction and the level of anti-oxLDL antibodies influence CV risk (Adv Clin Exp Med 2016, 25, 1, 43–50).

Key words: intima-media thickness, oxidized low-density lipoprotein cholesterol, anti-oxidized low-density lipoprotein cholesterol antibodies, rheumatoid arthritis, systematic coronary risk evaluation.

It is well documented that patients with rheumatoid arthritis (RA) have shortened lifespans compared to the general population [1] and that the mortality gap between RA patients and the general population is continuously widening [2]. Many publication have demonstrated that the high premature mortality in the RA population can be at least partially attributed to cardiovascular disease.

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Atherosclerosis is a slowly progressing inflammatory disease leading to severe CV complications. However, it remains asymptomatic for many years before the first CV event occurs. One of the first stages of subclinical atherosclerosis is thickening of the intima-media complex [18]. It has been demonstrated that thickening of the carotid intima-media complex measured by B-mode ultrasound is associated with future CV events [19]. Carotid intima-media thickness (IMT) and carotid plaques can be regarded as CV risk factors in non-rheumatic individuals as well as in RA patients [20]. The European League against Rheumatism (EULAR) recommends using a modified Systematic Coronary Risk Evaluation (SCORE) to determine the 10-year risk of fatal CVD in RA patients [5].

The aim of this study was to look for non-classical risk factors that can at least partially explain the increased CVD risk in RA patients. The risk of CV events was assessed on the basis of IMT measurements and a modified SCORE. Disease activity, the presence of rheumatic nodules and autoantibodies, the oxidation of LDL and the production of anti-oxLDL antibodies were analyzed.

**Material and Methods**

**Participants**

The study was conducted on 37 patients (34 females, 3 males) aged 20–72 years (mean age 50.1 years) with an established diagnosis of rheumatoid arthritis. The inclusion criteria for the RA group were an established diagnosis of RA and age above 18 years. Exclusion criteria for the RA group included overlap syndromes, peripheral arterial disease (PAD), coronary heart disease (CHD), heart failure, cardiomyopathy, a history of cerebral stroke, severe renal dysfunction, chronic liver failure, chronic or acute infections and a history of malignant neoplasm.

As a control group 24 healthy volunteers (21 females, 3 males) aged 34–62 years (mean age 48.1 years) were enrolled. The exclusion criteria for the control group included RA or any other arthritis or connective tissue disease, cardiovascular diseases, any other organ system disease, chronic or acute infections and a history of malignant neoplasm. The volunteers were also excluded if physical examination revealed any clinically significant abnormalities.

The study was approved by the Wroclaw Medical University ethics committee (05.05.2010; No. KB-153/2011) and written informed consent was obtained from all participants prior to their inclusion in the study.

**Clinical Data**

The data for the analysis were obtained from medical histories, physical examinations, laboratory tests and ultrasound examinations of the carotid
arteries. The participants’ data were collected, including age, sex, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), smoking habits, medical history, and current medication. Hypertension was defined as an SBP greater than 140 mm Hg and/or DBP greater than 90 mm Hg on repeated measurements, and/or receiving antihypertensive treatment. Hyperlipidemia was defined as total cholesterol (TC) concentration greater than 5.2 mmol/L and/or low-density lipoprotein cholesterol (LDL-C) concentration greater than 3.5 mmol/L, and/or high-density lipoprotein cholesterol (HDL-C) concentration lower than 0.9 mmol/L for males and 1.3 mmol/L for females, and/or triglycerides (TG) concentration greater than 2.3 mmol/L, and/or being under hypolipidemic treatment.

In the RA group, disease activity was measured using the Disease Activity Score 28 (DAS28). High disease activity was defined as a DAS28 score ≥ 5.1, low disease activity was defined as DAS28 < 3.2, and remission as DAS28 < 2.6.

**SCORE Risk Estimation**

The SCORE for the control group and the EULAR-modified SCORE for the RA group were calculated to determine the 10-year risk of fatal CV disease [5, 21]. The SCORE assessment was based on gender, age, smoking, systolic blood pressure and atherogenic index (total cholesterol/high-density lipoprotein cholesterol) [5, 21]. In accordance with EULAR’s recommendation, a multiplication factor of 1.5 was used when a patient with RA met two of the following three criteria: disease duration of more than 10 years, the presence of RF or anti-CCP antibodies, and/or the presence of extra-articular manifestations [5].

**Carotid Ultrasonography Examination**

The IMT in the common carotid artery (CCA) was measured according to accepted methodology [22], using a GE Vivid 7 Dimension ultrasound device (General Electric) equipped with a 12-MHz linear-array transducer and an automatic protocol for IMT measurement applying gray-scale analysis. During the ultrasonography examination focal plaques in the extracranial carotid tree were also detected [22]. Plaques were defined as IMT ≥ 1.3 mm.

Salonen et al. observed that a mean carotid IMT > 0.70 mm was associated with increased risk of myocardial infarction (MI), and that the risk of MI increased 11% per 0.10 mm of IMT [23]. In the present study thickening of intima media was therefore defined as carotid IMT > 0.70 mm. IMT > 0.90 mm and the presence of plaques are predictors of CV events in RA patients and in the general population [20, 24]. In the present study high/very high CV risk was therefore defined as IMT > 0.90 mm and/or the presence of plaques.

**Laboratory Measurements**

A venous blood sample was collected from each participant under fasting conditions. TC, HDL-C, LDL-C and TG concentrations in both groups were measured in a certified commercial laboratory. In the RA group, the CRP level and ESR were measured as well. In the RA group, the presence of RF and anti-CCP antibodies was recorded. The serum concentration of oxLDL and anti-oxLDL antibodies was measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits (ox-LDL/MDA Ad ductELISA Kit and Anti ox-LDL ELISA Kit, Immundiagnostik AG, Bensheim, Germany). All the tests for each sample were performed according to the manufacturer’s instructions in random order by a technician who was unaware of which group the sample belonged to. The oxidized LDL fraction in LDL was calculated.

**Statistical Analysis**

All numeric variables were expressed as mean ± standard deviation (SD), and categorical data were expressed as a number (n) and percentage in parentheses. Data were tested for normal distribution using the Kormogorov-Smirnov test. A univariate analysis of normally distributed continuous numerical variables was done with Student’s t test, while the χ2 test was used for categorical variables. Correlations were assessed by the Pearson correlation analysis. All tests of significance were two-tailed. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed with STATISTICA software, v. 10 (StatSoft, Inc, Tulsa, OK, USA).

**Results**

The characteristics of the study population are shown in Table 1. There was no difference between the groups in terms of age or the proportion of women. In the RA group, hypertension was observed in 38% of the patients, while in the control group no one was diagnosed as hypertensive. The rate of cigarette smoking was equivalent in the two groups, and RA patients were significantly
less likely to have elevated levels of LDL cholesterol. Although the mean carotid IMT was equivalent in the two groups, SCORE results were significantly higher in the RA group. In the RA group, significant elevation of the oxLDL fraction was also found.

In both the RA group and in the controls, significant correlations between the mean carotid IMT and age were detected (RP = 0.65, p < 0.0001 and RP = 0.54, p = 0.008, respectively).

Among the RA anti-CCP-positive patients, the carotid intima-media complex was significantly thicker than among the anti-CCP-negative ones (0.58 ± 0.18 mm vs. 0.38 ± 0.23 mm, p = 0.039) and the SCORE was elevated (4.7 ± 3.9 vs. 0.9 ± 1.3, p = 0.049). There was no association between IMT and ESR, CRP or the presence of RF.

In the RA group, significant correlations were found between SCORE results and the mean carotid IMT (RP = 0.34, p = 0.040), DAS28 (RP = 0.42, p = 0.011), ESR (RP = 0.35, p = 0.036), and disease duration (RP = 0.52, p = 0.002). Among the RA patients that were in remission or had low disease activity (11 patients), the SCORE was significantly lower than in RA patients with moderate to high disease activity (24 patients); (0.8 ± 1.8 vs. 4.0 ± 4.0, p = 0.016).

The mean carotid IMT and SCORE were equivalent in RA patients with and without rheumatoid nodules.

Table 1. Clinical characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 24)</th>
<th>RA patients (n = 37)</th>
<th>P*a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females*</td>
<td>21 (87.5)</td>
<td>34 (91.9)</td>
<td>ns.</td>
</tr>
<tr>
<td>Age, years*</td>
<td>48.1 ± 7.6</td>
<td>50.1 ± 15.1</td>
<td>ns.</td>
</tr>
<tr>
<td>Concomitant diseases*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• hypertension</td>
<td>0</td>
<td>14 (37.8)</td>
<td>0.0003</td>
</tr>
<tr>
<td>• hyperlipidemia</td>
<td>18 (75.0)</td>
<td>21 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Smokers*</td>
<td>6 (25.0)</td>
<td>9 (24.3)</td>
<td>ns.</td>
</tr>
<tr>
<td>SCOREb,z</td>
<td>0.8 ± 1.2</td>
<td>3.1 ± 3.7</td>
<td>0.005</td>
</tr>
<tr>
<td>RA duration, yearsz</td>
<td>NA</td>
<td>12.8 ± 10.3</td>
<td></td>
</tr>
<tr>
<td>Autoantibodies*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• RF</td>
<td>NA</td>
<td>25 (67.6)</td>
<td></td>
</tr>
<tr>
<td>• anti-CCP</td>
<td>NA</td>
<td>20 (54.1)</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid nodules*</td>
<td>NA</td>
<td>11 (29.7)</td>
<td></td>
</tr>
<tr>
<td>DAS28z</td>
<td>NA</td>
<td>4.3 ± 1.45</td>
<td></td>
</tr>
<tr>
<td>RA treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• DMARDs*</td>
<td>NA</td>
<td>32 (86.5)</td>
<td></td>
</tr>
<tr>
<td>• Biologics*</td>
<td>NA</td>
<td>11 (29.7)</td>
<td></td>
</tr>
<tr>
<td>• GCs*</td>
<td>NA</td>
<td>28 (75.7)</td>
<td></td>
</tr>
<tr>
<td>• mean daily dose of GCs*, mg*</td>
<td>NA</td>
<td>7.3 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Mean IMT, mm*</td>
<td>0.57 ± 0.12</td>
<td>0.54 ± 0.18</td>
<td>ns.</td>
</tr>
<tr>
<td>IMT &gt; 0.90 mm and/or carotid plaques*</td>
<td>6 (25.0)</td>
<td>8 (21.6)</td>
<td>ns.</td>
</tr>
<tr>
<td>• carotid plaques*</td>
<td>2 (8.3)</td>
<td>6 (16.2)</td>
<td></td>
</tr>
<tr>
<td>TC, mmol/L*</td>
<td>6.15 ± 1.10</td>
<td>5.65 ± 1.26</td>
<td>ns.</td>
</tr>
<tr>
<td>LDL-C, mmol/L*</td>
<td>3.91 ± 0.95</td>
<td>3.31 ± 1.04</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL-C, mmol/L*</td>
<td>1.69 ± 0.33</td>
<td>1.66 ± 0.50</td>
<td>ns.</td>
</tr>
<tr>
<td>TG, mmol/L*</td>
<td>1.20 ± 0.49</td>
<td>1.53 ± 0.66</td>
<td>0.04</td>
</tr>
<tr>
<td>oxLDL fraction of LDL-C, %*</td>
<td>0.014 ± 0.006</td>
<td>0.029 ± 0.033</td>
<td>0.04</td>
</tr>
<tr>
<td>Anti-oxLDL antibodies, U/mL*</td>
<td>50700 ± 10500</td>
<td>52100 ± 21200</td>
<td>ns.</td>
</tr>
</tbody>
</table>

* Data is presented as number (percentage); z data is presented as mean ± standard deviation; a RA group vs. control group; b EULAR-modified SCORE in RA group [7]; c counted as a dose of prednisone.
In RA patients with carotid plaques, the ox-LDL fraction was significantly higher than in patients without carotid plaques (0.055 ± 0.070 % vs. 0.022 ± 0.018%, p = 0.033) (Fig. 1).

In the RA group, a significant negative correlation was found between the mean carotid IMT and the serum concentration of anti-oxLDL antibodies (RP = -0.38, p = 0.02) (Fig. 2). In addition, in RA patients with thickening of the carotid intima-media complex (IMT > 0.70 mm) the concentration of anti-oxLDL antibodies was significantly lower (37800 ± 1100 U/mL vs. 57600 ± 22300 U/mL, p = 0.009). In RA patients receiving glucocorticoids, a negative correlation was found between the concentration of anti-oxLDL antibodies and the daily prednisone dose (RP = -0.5, p = 0.007).

**Discussion**

In the present study, no significant differences between the RA group IMT and the control group IMT. This might be caused by the lipid abnormalities in the control group, as significant elevation in LDL-C concentration was noticed in the controls. However, the 10-year risk of fatal CV disease was significantly higher in the RA group. A significantly increased risk of CV events among RA patients has been described by other authors [7].

In the present study, contrary to the reports of other authors, severe thickening of intima-media complex (IMT > 0.90 mm) and/or plaques were detected only in 21.6% of the RA patients. The reason for this discrepancy may be the fact that RA
patients were enrolled in this study without clinical manifestation of atherosclerosis. Additionally, the cohort seems to be younger than groups analyzed in other studies [25, 26]. A younger RA group might also be responsible for the lower mean IMT than in other reports [25]. Although Ahmed et al. analyzed an equally young group of RA patients, severe atherosclerosis was more frequent in their cohort [27]. This might be due to the fact that in 12.5% of the RA patients assessed by Ahmed et al. were diabetic, as opposed to none in the present study; and in 22.5% of the RA patients in those authors’ study reported a significant family history of CV diseases, while patients with a significant family history of CVD were excluded from the present study.

As expected, age, a known CV risk factor, was strongly associated with atherosclerosis in both the RA patients and the controls. The data from the study participants is similar in this respect to the general world population.

In the present study, thickening of the intima-media complex and increased SCORE results were observed in anti-CCP-positive RA patients. These results support the thesis that anti-CCP antibodies are among the non-classical CV risk factors in RA patients [5]. Cambridge et al. reported that anti-CCP antibodies might increase the risk of coronary heart disease even in patients without RA [28].

In the RA group in the present study there were associations between the 10-year risk of fatal CV disease (SCORE) and inflammation (ESR), disease activity (DAS28) and disease duration. Ahmad et al. also reported associations between subclinical atherosclerosis and inflammation, disease duration and the number of involved joint areas [29]. In the present study, in RA patients with low disease activity or remission the SCORE results were significantly lower than in patients with moderate to high disease activity. This supports the thesis that the best way to reduce CVD occurrence in RA patients is RA treatment leading to disease control and remission [5].

In RA rheumatoid nodules are among the clinical manifestations of more aggressive disease, but the present study did not find any association between the presence of rheumatoid nodules and ultrasonographic evidence of carotid atherosclerosis (IMT or plaques) or CV risk (according to the modified SCORE). Similar results have been reported by Galarza-Delgado et al. [26].

In the present study a higher fraction of oxLDL was detected in the RA patients than in the controls, and in the RA patients with carotid plaques compared to those without plaques. Ajeganova et al. reported higher oxLDL concentrations in RA patients who experienced a subsequent CV disease [30]. Elevated oxLDL may link chronic inflammation in RA patients with acceleration of atherosclerosis and an increased risk of CV disease in this population. The negative correlation between the concentration of anti-oxLDL antibodies and IMT suggest that these antibodies may be protective and reduce the risk of CVD. Some authors have reported an inverse correlation between IMT and anti-oxLDL antibodies in healthy subjects without any CVD [31]. However, the role of anti-oxLDL antibodies in the development of atherosclerotic changes needs further research. The current authors plan to further investigate the relationship between anti-oxLDL antibodies and CV complications in RA patients by increasing the size of the RA group and following them up.

The present study demonstrated that in RA patients disease activity, ESR, disease duration, the presence of anti-CCP antibodies, a high oxLDL fraction and a low level of anti-oxLDL antibodies may influence the risk of CVD. No influence of rheumatoid nodules on the risk of CVD was detected.

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References
Cardiovascular Risk Factors in RA


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